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Fulminant Demyelinating Encephalomyelitis Associated With Productive HHV-6 Infection in an Immunocompetent Adult

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Human herpesvirus 6 (HHV-6), the etiologic agent of roseola in young children, has been reported to be detectable in the brain of many neurologically normal adults, although regional localization to plaques of multiple sclerosis has also been demonstrated. Large amounts of this virus were present in multifocal demyelinating white matter lesions of fulminant encephalomyelitis with seizures in a 21-year-old woman with normal immune parameters. Brain biopsy after 3 weeks of neurologic deterioration revealed a viral etiology by light and electron microscopy; the virus was identified as HHV-6 by immunohistochemistry and by polymerase chain reaction (PCR) amplification in biopsy and autopsy specimens. ***J. Med. Virol. 52:301–308, 1997.***

KEY WORDS: human herpes virus 6; encephalomyelitis; demyelination;

INTRODUCTION

Human Herpesvirus-6 (HHV-6) has been identified [Yamanishi et al., 1988] as the causal agent of roseola in children. Analysis of population antibody profiles indicates nearly universal exposure to the virus by 1 to 3 years of age. Most of these infections in infancy resolve spontaneously, but in a few roseola patients, the virus causes febrile seizures, meningoencephalitis, and encephalopathy [Asano, 1992]. HHV-6 has been detected postmortem by polymerase chain reaction amplification in many apparently neurologically normal adult human brains [Luppi et al., 1994]. Two case reports have shown gray and white matter disease secondary to HHV-6 infection by immunohistochemistry in immunosuppressed individuals, such as an HIV-positive infant [Knox et al., 1995], and a bone marrow transplant recipient [Dobryski et al., 1994]. Recently, a study of 88 multiple sclerosis patients indicated that HHV-6 immunohistochemically localized in and around demyelinated white matter plaques [Challoner, 1994]. The patient of the present report was initially thought to have Devic's variant of multiple sclerosis, by clinical presentation and imaging characteristics of the multifocal demyelinating lesions. She demonstrated no immune deficiency after extensive studies. This is the first report of HHV-6 encephalomyelitis in an immunocompetent adult.

CASE HISTORY

A 21-year-old woman with a recent history of upper respiratory infection presented to the emergency room with a 2-day history of progressive loss of vision in the left eye, accompanied by deviation of the eye to the left, unsteadiness of gait, decreased left-sided sensation, and difficulty pronouncing words. She was alert and oriented, but slightly dysarthric. She had no light perception in her left eye, but showed a consensual pupillary response to light shone on the right side with a visual acuity of 20/200; vertical nystagmus, right sixth cranial nerve palsy, and right facial droop. Motor strength was normal, and sensory exam demonstrated decreased light touch on the left arm and leg. She exhibited dysmetria on her left side and fell to the left side on Romberg. She was a recent immigrant to Los Angeles from Mexico, single, and was employed in domestic childcare full-time.

MRI showed concentric, hypodense lesions with no mass effect in the dorsal pons, internal capsule, and in the parietal-occipital lobe extending into the optic ra-

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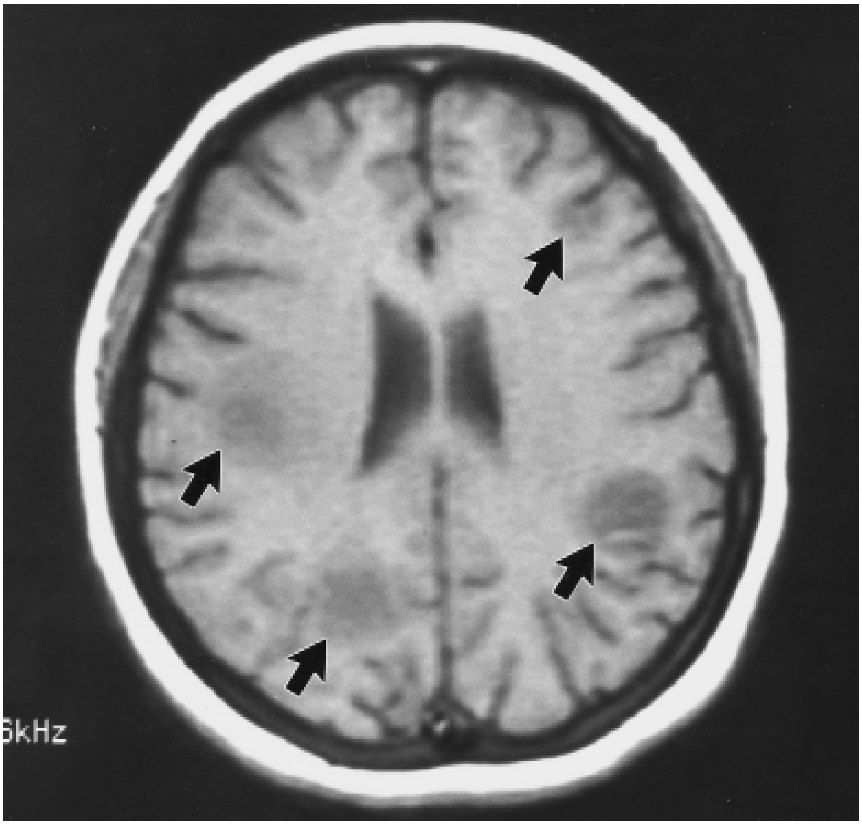

Fig. 1. Proton-density weighted MRI scan from hospital day 15 showing bilateral, rounded, hypodense lesions of various sizes in the deep white matter (arrows).

diations (Fig. 1). The lesions became hyperintense on T-2 weighted imaging but did not enhance with Gadolinium. The patient was afebrile, with white cell count of 11,400 with a normal differential. Electrolytes, BUN, creatinine, and coagulation parameters were normal. HIV-1 ELISA was negative initially, and again on hospital day 18. CD-4 count was 890 on hospital day 25. Initial lumbar puncture showed opening pressure of 22 cm, glucose 44, protein 16, 9 WBC, and 20 RBC, with a lymphocytic predominance. CSF was culture negative for bacteria, fungi, Herpes simplex (HSV), and cytomegalovirus (CMV). Mumps and measles antibodies were not detected. Serological tests for Listeria, toxoplasma, and cryptococcus were negative. Mycoplasma IgG was positive, but IgM was negative. CSF IgG and IgG synthesis rates were increased, and oligoclonal banding was observed.

Her hospital course was complicated by rapidly progressive blindness, vomiting, seizures, and then decreased level of consciousness with absence of response to verbal stimuli. She was treated since hospital day number 3 with intravenous steroids, and completed a 10-day course of acyclovir and broad spectrum antibacterial antibiotics. Repeat LP showed increasing leukocytosis, and MRI showed progression of white matter disease including large lesions in the pons and medulla. With a presumptive diagnosis of Devic's disease, she underwent three cycles of plasmapheresis (1 plasma volume each), with no apparent benefit. Brain biopsy of the most advanced parietal occipital lesion was performed on day 20, after 10 days of acyclovir therapy. After the diagnosis of viral encephalomyelitis, she continued to receive IV acyclovir without steroids, with stabilization of the size of her cerebral lesions. She did not receive gancyclovir or Foscarnet, agents shown in other studies to have in vitro activity against HHV-6 [Streicher et al., 1988; Russler et al., 1989], since a specific viral etiology was not established and HHV-6 was not considered in the differential diagnosis because of her normal immune status. She died in week 5 of hospitalization, without neurological improvement, from pulmonary emboli. Serum was obtained and sent for HHV-6 IgG and IgM, but was negative. Postmortem cultures of brain lesions for HSV and CMV were negative, and frozen tissues analysed for HSV, VZV, and JC virus by PCR techniques were also negative for these viruses.

Autopsy showed bilateral pulmonary emboli, and thrombi in the lower extremity veins. No evidence of lymphoproliferative disorder, other neoplasia, or bone marrow suppression was apparent. Sections of the brain after fixation disclosed discrete, slightly yellowed, gelatinous lesions surrounded by edema confined to the subcortical and deep white matter in the cerebrum (Fig. 2). Similar white matter lesions were present in the pons, midbrain, cervical spinal cord, and

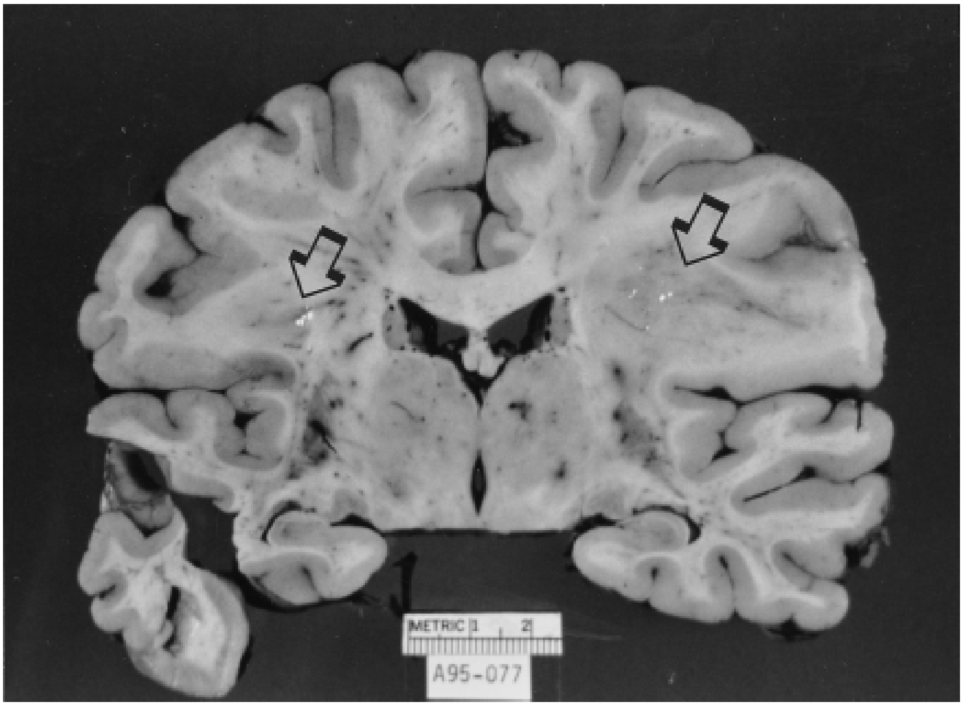

Fig. 2. Coronal section of post mortem brain demonstrating gelatinous, relatively well-circumscribed, slightly sunken lesions in the deep frontal white matter (arrows).

optic nerve. Well-demarcated grey, plaque-like lesions or periventricular foci of myelin deterioration were not found.

MATERIALS AND METHODS

Specimens from a parietal lobe brain biopsy performed by open craniotomy on the 20th hospital day, and autopsy performed 4 hours postmortem on the 38th hospital day, were examined by light and electron microscopy. For light microscopy and immunohistochemistry, tissue blocks were fixed in 10% formalin for 6 hours after biopsy, and the autopsy brain was fixed 10 days in neutral buffered formalin before coronal sectioning. In addition to routine hematoxylin and eosin and luxol fast blue staining, immunohistochemistry by avidin-biotin conjugated horse-radish peroxidase (HRP) antispecies technique for antibody localization of glial fibrillary acidic protein, leukocyte markers CD43 (Leu22), CD20 (L26), IgG, IgM, macrophage markers lysozyme and Kp-1, and viral antigens HSV I and II, EBV-LMP1, and CMV (all antibodies Dako, Carpinteria, CA) was performed on biopsy and autopsy blocks. Immunohistochemical localization of HIV p-24 (DuPont, Wilmington DE), and structural protein p101 of HHV-6 variant B (Biometria, Washington, DC) were performed by overnight incubation with primary antibody, followed by localization with HRP-labelled rabbit antimouse IgG, and HRP-labelled swine antirabbit IgG antibody (Dako) [Cornford et al., 1992]. Antibody localization was achieved with 3,3′ diaminobenzidine chromogen for all antibodies.

For electron microscopy, biopsy and 4 hour postmortem samples were fixed in 2.5% glutaraldehyde in 0.1 M pH 7.2 phosphate buffer, followed by 1 hour postfixation in 1% OsO4 in the same buffer and embedment in Medcast resin. Thin sections were stained with lead citrate and uranyl acetate. Micrographs were taken on a Hitachi 6 electron microscope operating at 75 kV.

The polymerase chain reaction (PCR) procedure was performed as reported previously [Vinters, 1993; Sanders, 1996]. Paraffin sections from the midbrain and pons (20 × 10 um thickness) were placed in a microfuge tube and dissolved by Histoclear. The tissue pellet was digested with Proteinase K. The DNA was precipitated and quantitated. 0.5 to 1 ug of extracted DNA was used as a template for PCR. Primers specific for coding regions of HSVI, HSVII, CMV, EBV, VZV, and HHV-6 were used as described, and specimens were run in triplicate. Optimal annealing temperature for each primer was determined empirically using the Stratagene Gradient Robocycler (TM). Reaction ingredients consisted of 200umol/L of each deoxynucleotide triphosphate (dNTP), 100 pmol/L of each primer, 0.01% acetylated bovine serum albumin, 1.5 mmol/L MgCl2, 50 mmol/L KCl, 10 mmol/L Tris, and 0.1% Triton X-100 in a reaction volume of 100ul. One unit of Taq polymerase was added in a modified “hot start” procedure. The reaction was thermocycled, and amplification was performed for 40 cycles. To identify false positive results, 25% reagent blank controls were included with each PCR thermocycling batch. Amplified viral sequences were detected by Southern blotting.

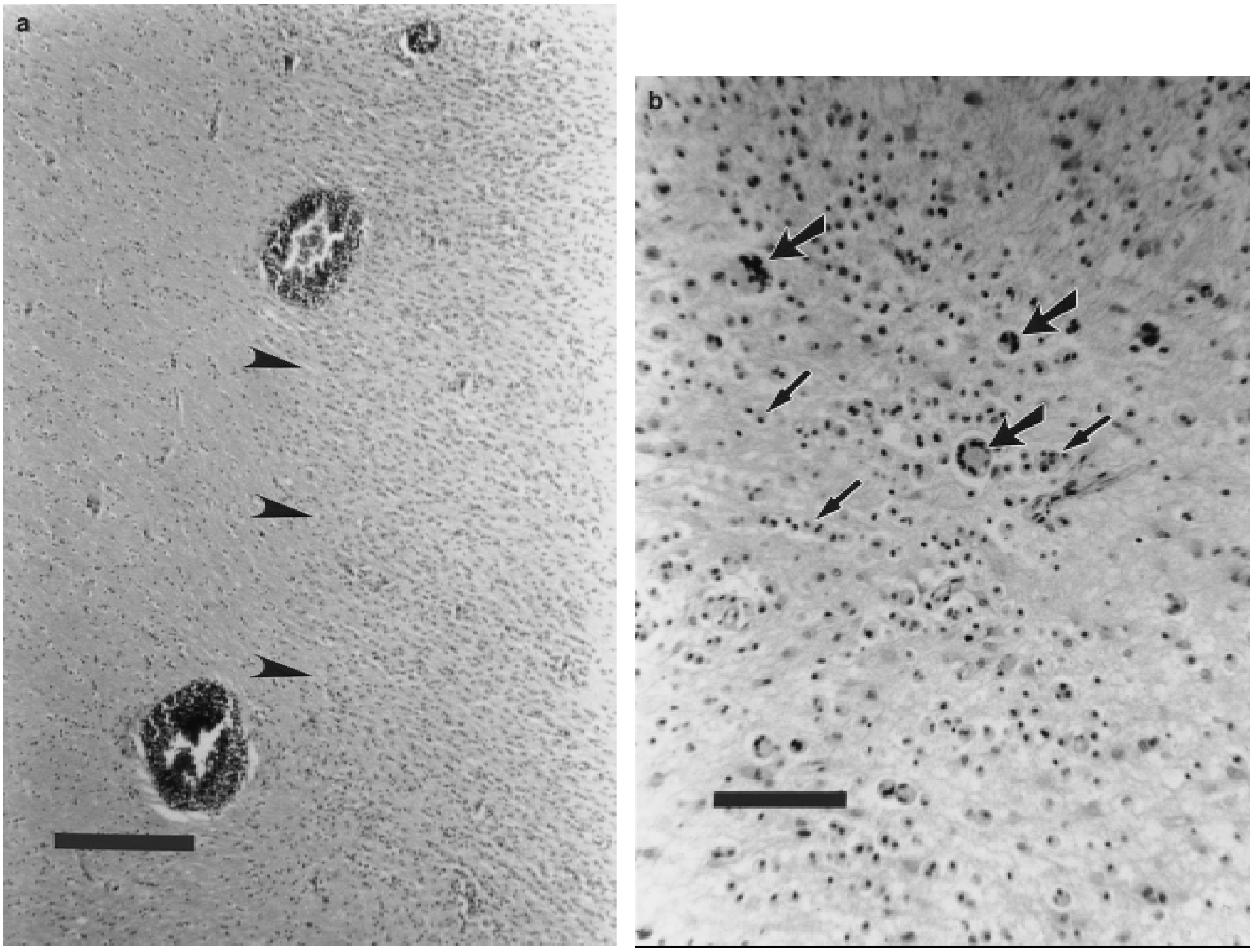

Fig. 3. **a**: Hematoxylin and eosin stained section of the edge of a large, well-developed lesion in the frontal white matter from autopsy material. There is prominent perivascular lymphocytic cuffing and and a discrete border between normal brain and area of infection (arrowheads) Magnification bar = 500 microns × 40. **b**: Higher magnification of the central portion of the lesion of (a) shows sheets of macrophages (small arrows) and gliosis. Some enlarged astrocytes (immunohistochemically positive for glial fibrillary acidic protein) have multiple nuclei or fragmented nuclei, resembling mitotic figures (large arrows). Magnification bar = 100 microns. × 200.

RESULTS
Light Microscopy

Lesions from different parts of the brain appeared similar histologically. There was perivascular lymphocytic cuffing at the edges of the white matter lesions, with no evidence of vasculitis, fibrinoid necrosis, or petechial hemorrhages (Fig. 3a). Centrally, most lesions showed nearly complete demyelination, with short, vacuolated residual segments of myelin sheaths and few residual oligodendrocytes. The lesions were infiltrated densely by macrophages and by numerous hypertrophic astrocytes. Some of the GFAP-positive astrocytes had fragmented nuclei (Fig. 3b). The most advanced white matter areas were largely devoid of oligodendrocytes, but retained intact axons on Bodian staining. Adjacent to the areas of complete demyelination, microglial cells and mature lymphocytes infiltrated into the edematous neuropil.

Immunohistochemistry was performed on the biopsy and on two lesions from the autopsy material. Lymphocytic cuffs at the edge of the lesions were principally T cells, with about 50% T-helper cells. IgM and IgG-positive B cells were scattered in small numbers through areas of partial demyelination. HHV-6 early protein antigen localized within oligodendrocytes in the biopsy specimen (Fig. 4a). HHV-6 antigen localized in nucleus and cytoplasm, with cytoplasmic staining more marked than nuclear staining in most infected cells. In more demyelinated lesions in autopsy tissue, hypertrophic astrocytes and rounded, anucleate cell fragments localized virus strongly (Fig. 4b,c). Neuropil showing normal myelination in the autopsy sections did not localize HHV-6. Rare lymphocytes in the perivascular cuffs localized HHV-6. HIV, CMV, EBV, or HSV I and II antigens were not localized in biopsy or autopsy sections.

Ultrastructural study showed that rounded, anucleate cell fragments in advanced lesions were virus-filled spheres of cytoplasm. These spheres contained mitochondria, and occasionally had intermediate filaments

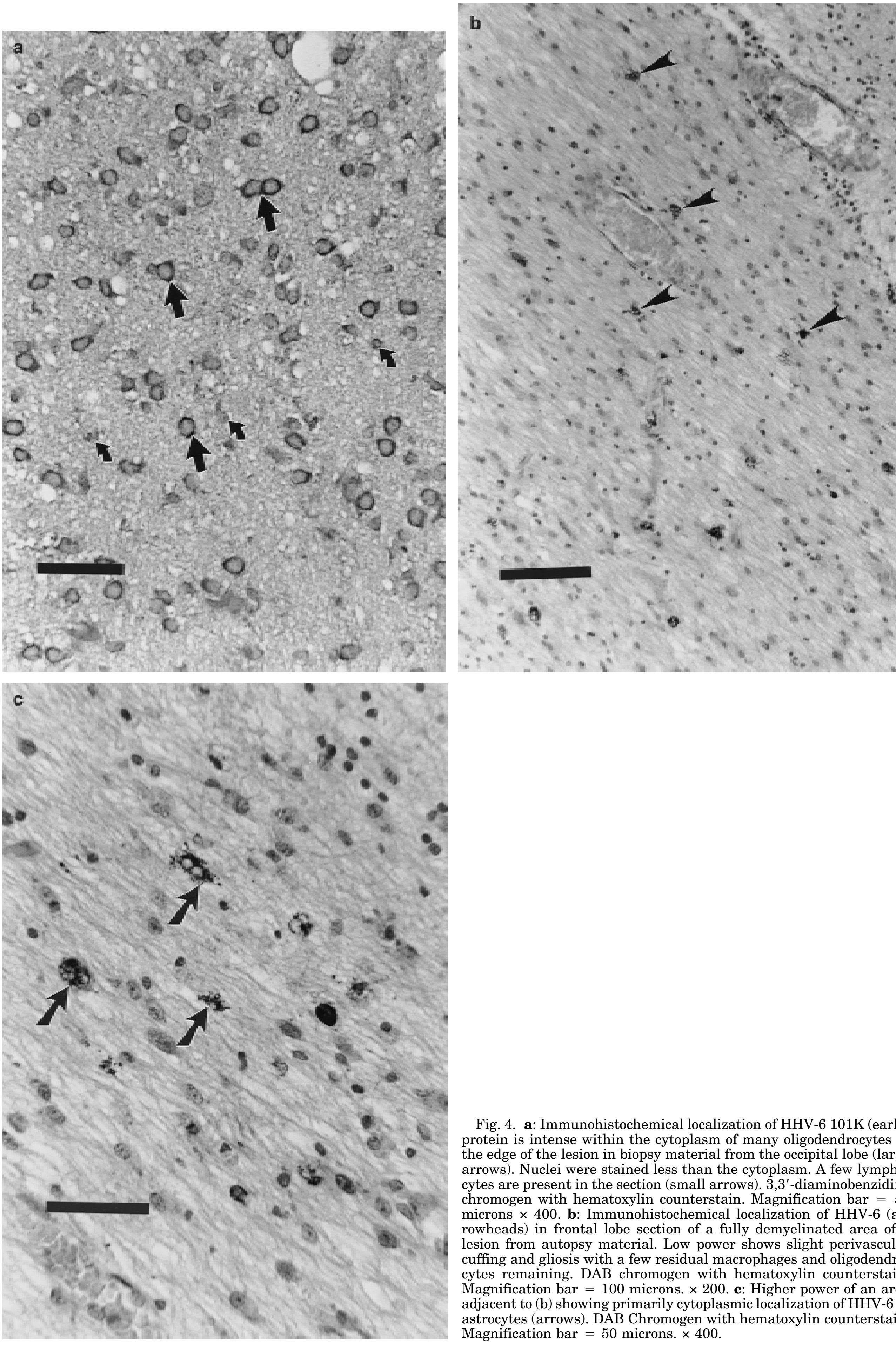

Fig. 4. **a**: Immunohistochemical localization of HHV-6 101K (early) protein is intense within the cytoplasm of many oligodendrocytes at the edge of the lesion in biopsy material from the occipital lobe (large arrows). Nuclei were stained less than the cytoplasm. A few lymphocytes are present in the section (small arrows). 3,3′-diaminobenzidine chromogen with hematoxylin counterstain. Magnification bar = 50 microns × 400. **b**: Immunohistochemical localization of HHV-6 (arrowheads) in frontal lobe section of a fully demyelinated area of a lesion from autopsy material. Low power shows slight perivascular cuffing and gliosis with a few residual macrophages and oligodendrocytes remaining. DAB chromogen with hematoxylin counterstain. Magnification bar = 100 microns. × 200. **c**: Higher power of an area adjacent to (b) showing primarily cytoplasmic localization of HHV-6 in astrocytes (arrows). DAB Chromogen with hematoxylin counterstain. Magnification bar = 50 microns. × 400.

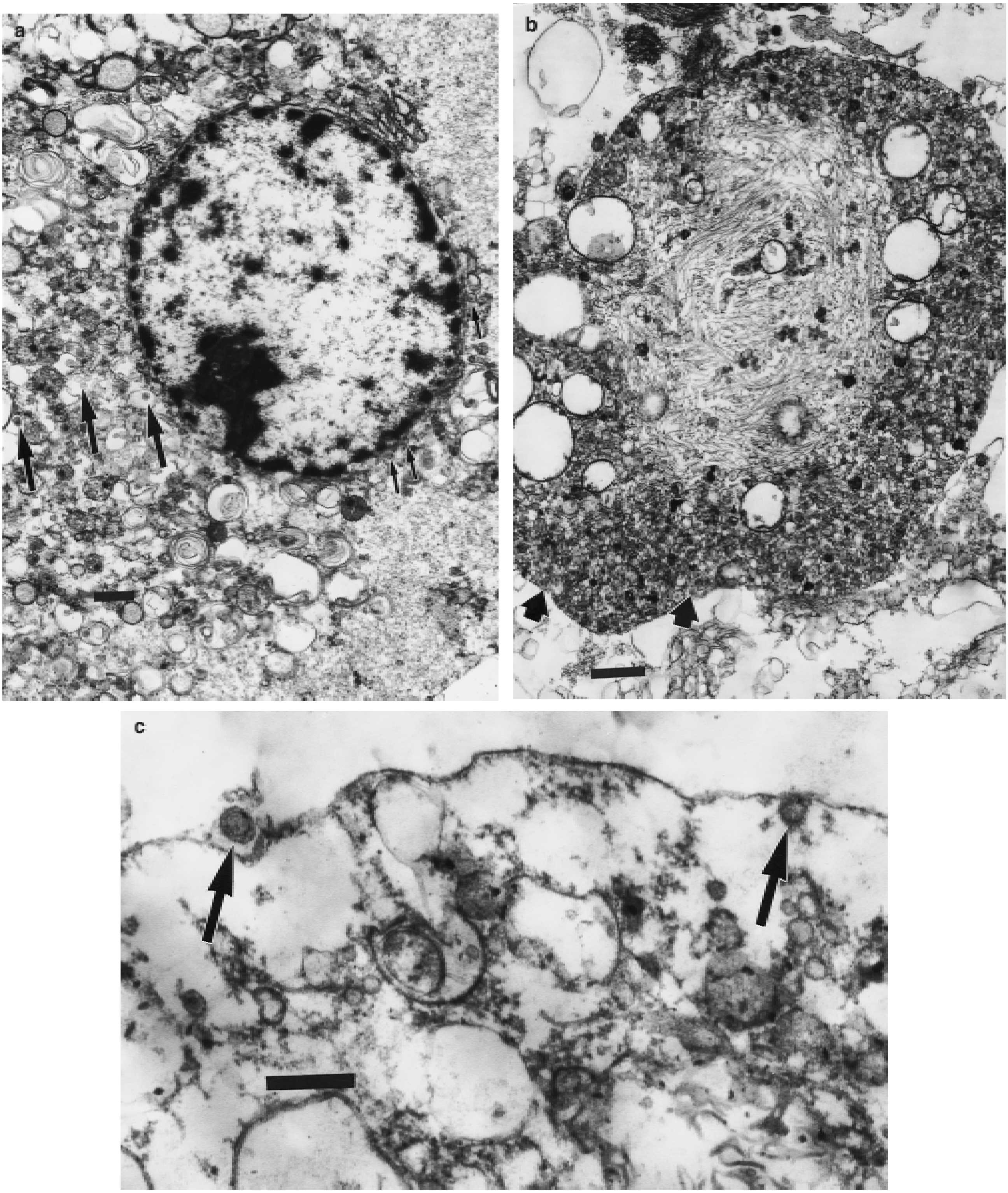

Fig. 5. **a**: Electron micrograph of an oligodendrocyte with early HHV-6 infection, showing viral particles budding into vesicles formed by the nuclear membrane (small arrows) and similar particles within the cytoplasm (large arrows). Magnification bar = 1 micron. × 10,000. **b**: Electron micrograph demonstrating the accumulation of masses of enveloped viral particles in the cytoplasm of an astrocyte. The nucleus was typically absent from cells with this amount of cytoplasmic virus. Many of the particles appear defective, with multiple, variable size, or no nucleocapsids, and variable envelope appearance. Rare viral particles, compared to the amount of virus within the cell, are budding from the plasma membrane (arrows). Magnification bar = 0.5 micron. × 7,000. **c**: Electron micrograph of enveloped viral particles budding from an oligodendrocyte. The diameter of the capsid and its icosahedral symmetry indicates a herpes virus. The complete viral particle is 140–170 nm in diameter. Magnification bar = 0.5 micron. × 30,000.

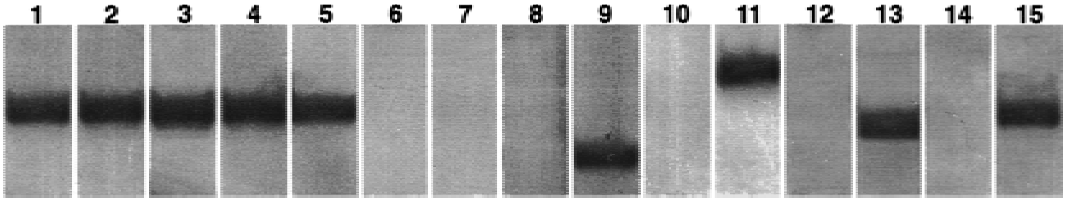

Fig. 6. Composite figure of PCR test for herpesviridae. **Lanes 1,2**: HHV-6 PCR from the biopsy. **Lanes 3,4**: HHV-6 PCR from the autopsy specimens. **Lane 5**: Positive control HHV-6 PCR showing a 384 bp amplicon. **Lanes 6,7**: HHV-6 PCR from negative control patients. **Lanes 8,9**: HSV PCR from the patient sample and positive HSV control. **Lanes 10,11**: CMV PCR from the patient sample and positive CMV control, respectively. **Lanes 12,13**: EBV PCR from the patient sample and positive EBV control, respectively. **Lanes 14,15**: VZV PCR from the patient sample and positive VZV control. The patient sample is positive for HHV-6 DNA and negative for HSV, CMV, EBV, and VZV by PCR.

typical of astrocytes, but no nuclear remnants. The masses of enveloped virus in the cytoplasm of these cells contain many empty envelopes, some multiple nucleoids, as well as structurally typical herpes virus (Fig. 5a) Some cells with evidence of viral infection were contiguous with myelin figures at the plasma membranes, and are therefore identified as oligodendrocytes. A sequence of viral production involving nuclear particles, blebbing of the outer nuclear membrane, enveloped virus within the cytoplasm or vacuoles, and release of virus from the plasma membrane of a few cells was evident (Fig. 5b,c). No viral capsids or enveloped virus were identified ultrastructurally in lymphocytes, macrophages, or endothelial cells.

The dimensions of the best formed (budding) viral particles were a nucleocapsid of 70–90 nm, with complete enveloped virus of about 140 nm to 170 nm in diameter (Fig. 5c). This agrees with the dimensions reported for the virus in B-lymphocyte culture [Biberfeld et al., 1987].

The amplified products of the PCR reaction were detected by Southern blotting, and the PCR gel is shown in Figure 6. Lanes 1 through 4, in which the patient specimen was run, exhibited a strong signal at 384 bp, which corresponded to the amplicon size between the primers of the HHV-6 control in lane 5. Two other patients with demyelinating disease were negative (lanes 6 and 7). No cross reactivity to HSV, CMV, EBV, or VZV was observed (lanes 8 through 15).

DISCUSSION

HHV-6 has previously been detected by immunohistochemistry in the brains of immunosuppressed individuals with encephalitis. The present case indicates, however, that this virus may cause rapidly expanding, multifocal demyelinating lesions in days to weeks, in an adult without overt immunosuppression. HIV serology was repeatedly negative, immunoglobulin and helper T-cells were at relatively normal levels late in the illness, and no other cause of immune abnormality was found on complete postmortem examination. The reason for the virulence of this virus in this person is not evident, unless she had no previous immunity to this very common childhood viral infection because of lack of exposure, or had encountered a significantly different strain. Her presentation with visual loss, multifocal demyelinating lesions, and other clinical criteria of neuromyelitis optica, or Devic's disease is very interesting, in light of the immunohistochemical localization of HHV-6 p-101 antigen to oligodendrocytes in multiple sclerosis plaques in the chronic form of the disease [Challoner et al., 1995]. It must be emphasized that the gross appearance of the brain was not consistent with multiple sclerosis, without periventricular foci, or typical grey, sharply defined, nonangular demyelinated plaques. However, it is possible that very early, days old initial lesions of multiple sclerosis might lack those features.

The tropism of HHV-6 for astrocytes and oligodendrocytes has not previously been demonstrated ultrastructurally. In this patient, enveloped viral particles were present in cytoplasmic masses in degenerating cells or perhaps astrocytic cell processes, as well as, more typical of herpes viruses, seen in small numbers moving through the cytoplasm after acquiring an envelope from the nuclear membrane. Both biopsy and autopsy specimens were obtained after treatment with acyclovir, which may have induced production of defective virus. The usual array of herpes simplex virus in cell culture nuclei shows much more regular nucleoids with capsids, but the ultrastructural appearance of the virus in the present case is comparable to published illustrations of Epstein-Barr Virus in hairy leukoplakia of the oral cavity [Zhang et al., 1988]. Ultrastructurally, the viral particles have a more attenuated envelope than depicted for HHV-6 in B-lymphocyte culture [Biberfeld et al., 1987], although the dimensions and cell budding process are comparable, as is the lack of nuclear paracrystalline capsids for this virus. The possibility of a viral coinfection with a virus not included in those probed for by PCR, or a defective virus with subnormal viability that causes cell death by host immune reaction rather than viral lysis should be considered in accounting for the ultrastructural appearance. However, immunohistochemistry supports the presence of a productive viral infection by HHV-6, because the structural protein 101K was strongly expressed.

HHV-6 should be considered in the differential diagnosis of acute demyelinating encephalomyelitis in immunologically competent adults. This patient was thought to have a rapidly progressive form of multiple sclerosis, but immunosuppressive measures appropriate for that diagnosis were ineffective in slowing the

progression of the radiologically evident lesions, prompting biopsy. The biopsy was not histologically typical for either multiple sclerosis or acute demyelinating encephalomyelitis; ultrastructural examination of the biopsy suggested the viral etiology in the absence of serological or CSF serologic evidence of a specific virus. While the patient in this case expired of medical conditions not directly related to progression of her central nervous system lesions, acyclovir may have been somewhat effective in stabilizing the rate of expansion of the lesions radiologically.

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